

Effect of Restricted Root Growth on Carbohydrate Metabolism and Whole Plant Growth of *Cucumis sativus* L.¹

Received for publication July 16, 1987 and in revised form January 27, 1988

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ABSTRACT

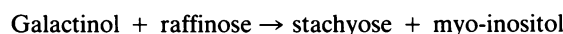
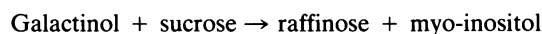
The effects of varied rooting volumes on root growth and source leaf carbohydrate metabolism were studied in greenhouse-grown cucumber (*Cucumis sativus* L. cv Calypso) plants. Plants were grown for 7 weeks in container volumes that ranged from 0.4 to 5.9 liters. Plants grown in the smaller containers exhibited less leaf expansion, lower root and shoot weight, and fewer lateral stems than plants grown in the 5.9 liter containers. Shoot/root ratio was not altered by the container volume, suggesting coordination of root and shoot growth due to rooting volume. Source leaf carbon exchange rates, assimilate export rates, and starch accumulation rates for plants grown in 0.4 liter containers were approximately one-half or less in comparison to those for plants grown in 5.9 liter containers. Starch concentrations per unit leaf area were maintained at high levels in source leaves of plants grown in 0.4 liter containers over the entire day/night cycle. Lower extractable galactinol synthase activities and higher galactinol concentrations occurred in leaves of plants grown in 0.4 liter container volumes. The reduced sink demand, induced by restricted root growth, may have led to increased starch concentrations and to a reduction in stachyose biosynthesis in cucumber source leaves.

Reduced root growth due to restricted soil volume has long been associated with reduced shoot growth. The reduction in shoot growth cannot be assumed to result from water and/or nutrient stress. Restricting the volume in which bean plant (*Phaseolus vulgaris* L.) (1, 2) and winter wheat (*Triticum aestivum* L.) (9) roots grew, under otherwise favorable environmental conditions, resulted in dwarf plants. Growth was reduced in terms of leaf area, stem size, and shoot dry weight.

Roots are recognized as metabolic sinks that influence the partitioning of photosynthetically fixed carbon (4). Formation of adventitious roots has been associated with increased carbon fixation rates (7). Conversely, partial excision of vegetative bean plant roots decreased photosynthesis and net assimilate export (3). Bean plants grown under reduced root volume conditions exported less assimilate export and accumulated higher concentrations of leaf starch during the photoperiod (2).

Photosynthetic rates and carbohydrate partitioning in source leaves of cucumber plants can be altered in response to sink demand associated with development of a single fruit (10). Source leaves of fruiting cucumber plants had higher photosynthetic and

assimilate export rates than those of vegetative plants (10). Cucumbers are one of several plants that export stachyose (10, 11, 19). Stachyose biosynthesis involves three steps in the formation of stachyose from sucrose (8):



Changes in activity of enzymes catalyzing these three reactions may in part regulate the proportion of photoassimilate available for export to actively growing sinks (10, 11). The present study was conducted to examine the effects of restricted root growth on carbohydrate metabolism and whole plant growth of cucumber. Additionally, the activity of Gal Syn,² the enzyme responsible for the first reaction above and a potential regulatory point in stachyose synthesis, was investigated.

MATERIALS AND METHODS

Plant Material. Cucumber seeds (*Cucumis sativus* L. cv Calypso) were germinated and grown in plastic 0.4, 1.3, 2.9, and 5.9 L containers. These volumes correspond to widths and depths of 10 × 8.5, 14 × 11.5, 20 × 15, and 25 × 19 cm, respectively. Seeds were sown directly into washed sand, and seedlings were thinned to one plant per pot upon germination. The experiment was carried out in a glass greenhouse during spring and summer. Minimum day and night temperatures were maintained at 27 and 21°C, respectively. Vines were supported with string and the aerial volume available for shoot growth was not restricted. Hoagland nutrient solution was delivered via an automated drip system several times during the photoperiod. Irrigation frequency was scheduled so that no visible water stress occurred for plants grown in the different container volumes. Only 1 cm of the sand surface was allowed to dry between irrigation cycles. Leachate was collected every 14 d for electrical conductivity and pH measurements. Plants were grown for 7 weeks.

Growth Measurements. Length and width of the second true leaf of seven plants were measured for 16 d after the leaf reached 3 cm in length. These measurements were used to nondestructively calculate increases in leaf area over the measurement interval (12). Number of nodes and laterals per plant were recorded on the day of sampling to document the morphological configuration of plants grown in each container.

Six source leaves from each of four plants per container volume were collected at the time of sampling (7 weeks). Leaves were dried in a forced air oven at 70°C, combined, weighed, and ground with a 20 mesh Wyley mill. Samples were analyzed for macro- and micronutrient content by the North Carolina De-

¹ Paper No. 11153 of the Journal Series of the North Carolina Agricultural Research Service, Raleigh, NC 27695-7601. Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the North Carolina Agricultural Research Service nor does it imply approval to the exclusion of other products that may be suitable. This work is a portion of a thesis submitted by the first author in the partial fulfillment for the PhD degree.

² Abbreviations: Gal Syn, galactinol synthase; AER, assimilate export rate ($\text{mg CH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$); CER, carbon exchange rate ($\text{mg CH}_2\text{O} \cdot \text{m}^{-2} \cdot \text{h}^{-1}$).

partment of Agriculture Plant Analysis Laboratory, Raleigh, NC. Roots and shoots from seven other plants from each container volume were collected and dried in a forced air oven at 70°C. Root and shoot tissue were weighed and placed in a muffle furnace for 24 h at 500°C. Upon cooling, loss of combustible organic matter was determined.

Carbon Exchange Rates. CER was measured in the most recently fully expanded source leaves located at the seventh node from the apex of the mainstem, hereafter called sample leaves (11). Measurements were made using an infrared gas analyzer (Anarad model AR600) and an ambient CO₂ concentration of 350 $\mu\text{l} \cdot \text{L}^{-1}$. Air was passed over adaxial and abaxial leaf surfaces through a 10 cm² leaf cuvette at a flow rate of 1.5 L $\cdot \text{min}^{-1}$. Differences in CO₂ concentration between air supplied to the leaf and exhausted air were measured. Two measurements were recorded during the middle of the sampling interval and were averaged. CER was calculated using the method of Hesketh and Moss (6) and expressed as mg CH₂O $\cdot \text{dm}^{-2} \cdot \text{h}^{-1}$.

Assimilate Export Rates. AER from source leaves was estimated using the method of Terry and Mortimer (18). Six leaf discs (1.38 cm² each) were removed from sample leaves at the beginning and end of the 6 h sampling interval (0900 and 1500 h) during the photoperiod. Leaf discs were freeze-dried and weighed to estimate the rate of dry weight change per unit area over the sampling interval.

A second group of plants was used for diurnal sampling. Six leaf disc samples from three adjacent source leaves (two 0.5 cm² discs/leaf) were removed every 3 h over a 24 h period. These leaf discs were handled as described above.

Carbohydrate Extraction and Analysis. Dried leaf discs were crushed with a glass rod, and then ground for 1 min in 3 ml of 80% (v/v) ethanol with a Brinkman Polytron Homogenizer (Brinkman Instruments, Westbury, NY). Samples were placed in a boiling water bath for 5 min, cooled, and centrifuged at 1000g for 10 min. The pellet was extracted two additional times with 3 ml 80% (v/v) ethanol. Supernatants from each extraction were retained, combined, and evaporated to dryness *in vacuo* at 45°C with a Buchler Evapo-Mix (Buchler Instruments, Fort Lee, NJ). Samples were resuspended in 0.5 or 1.0 ml distilled water and stored at -20°C.

Concentrations of the soluble sugars stachyose, raffinose, galactinol, and sucrose were analyzed using HPLC. The system included a Waters 6000A pump (Millipore, Waters Chromatography Division, Milford, MA), a Waters Sugar-Pak I column, and a Waters 401 refractive index detector connected to a strip chart recorder. Distilled water, at a flow rate of 0.5 ml $\cdot \text{min}^{-1}$, was used as the solvent. Column temperature was maintained at 75°C and was preceded by a Waters Bondapak C₁₈/Corasil guard and a set of anion and cation cartridges (Bio-Rad Laboratories, Richmond, CA, deashing guards). All guards were operated at an ambient temperature of 22°C. Twenty μl of sample were injected. Sugars were identified and quantified from retention time and peak heights of sugar standards.

Leaf residue remaining after ethanolic extraction was resuspended in 1 ml of 0.2 M KOH and boiled for 30 min. After cooling, samples were adjusted to pH 5.5 with 0.2 ml of 1 M acetic acid. Amyloglucosidase (Sigma Chemical), which had been dialyzed against 50 mM Na-acetate buffer (pH 4.5), was used to digest the gelatinized starch to glucose in a 55°C water bath for 60 min. The reaction was terminated by placing samples in a boiling water bath.

Released glucose was detected enzymically. An aliquot of sample was incubated for 30 min at 22°C in 0.5 ml of a reaction mixture containing 200 mM Hepes-NaOH buffer (pH 8.0), 10 mM MgCl₂, 10 mM DTT, 2 mM ATP, 2 mM NADP⁺, 2 units hexokinase (Sigma Chemical, Type VI from Bakers Yeast), and 2 units glucose-6-phosphate dehydrogenase (Sigma Chemical,

Type V from Bakers Yeast). A Lambda 3 Spectrophotometer (Perkins-Elmer Corp., Oak Brook, IL) was used to determine the reduction of NADP⁺ by glucose-6-phosphate dehydrogenase spectrophotometrically at 340 nm. Glucose concentrations were determined by comparisons to glucose standards.

Enzyme Extraction and Assay. Sample leaves were removed after the 1500 h sampling time for use in enzyme extraction and assay. Leaf extracts were prepared for Gal Syn assay by homogenizing (Brinkman Polytron Homogenizer, Brinkman Instruments, Westbury, NY) 2 gm fresh weight of shredded leaves at 0°C for 1 min in 50 mM Hepes-NaOH buffer (pH 7.0) and 10 mM DTT. Gal Syn activity was determined using an isotopic assay described by Handley *et al.* (5).

RESULTS

Plant Growth. Shoots and roots of plants grown in large containers grew more than plants grown in small containers (Fig. 1, A and B). Shoot growth was positively correlated with root growth ($r = 0.95$, $\alpha = 0.01$), suggesting that shoot/root ratio was unaffected by container volume (Fig. 2). Increased shoot and root growth were associated with greater dry weights of source leaves (Fig. 1C).

Cucumber plants grown in the four container volumes had different shoot morphology in terms of leaf size and lateral stems. Plants grown in 5.9 L containers had twice the number of lateral stems per plant as plants grown in 0.4 L containers (Fig. 1D). Additionally, leaf expansion (ending leaf area-beginning leaf area) over a 16 d time interval was reduced for plants with restricted root growth. Leaf expansion of plants grown in 0.4 L containers was only 60% of the leaf area of plants grown in 5.9 L containers (211.0 ± 9.3 and 371.3 ± 14.0 cm², respectively).

Electrical conductivity and pH measurements of the leachate from plants growing in different sized pots did not differ over the 7 week growing period and were within ranges acceptable for assuming that nutrient availability was not a factor limiting plant growth (data not shown). Foliar nutrient concentrations of essential macro- and micronutrients did not vary appreciably for cucumber plants grown in the different container volumes (Table I). Nitrogen, phosphorous, and potassium were present in lower concentrations in foliage from plants grown in the 0.4 L in comparison to those grown in larger containers. Also, foliar calcium and magnesium concentrations tended to decrease with increasing container size. Nevertheless, concentrations of all nutrients in the four treatments were within the range considered optimum for plant growth (14, 17).

Photosynthesis and Carbohydrate Partitioning. Photosynthesis rates and the partitioning of carbon were influenced by restricted root volume. Lowest rates of carbon fixation and photoassimilate export were measured in source leaves of plants grown in 0.4 L container volume (Fig. 3, A and B). CER *versus* AER in cucumber source leaves was positively correlated ($r = 0.76$, $\alpha = 0.01$). In general, both CER and AER increased with container size.

Leaf sucrose and starch status changed dramatically over the photoperiod for all cucumber plants, except those grown in 0.4 L containers. At the beginning of the sampling interval (0900 h) sucrose concentrations were generally higher in source leaves of plants grown in 0.4 L containers in comparison to those plants grown in larger containers (Fig. 3C). Starch concentrations at 0900 were approximately the same in source leaves of plants grown in each of the container volumes (Fig. 3D). Concentrations of sucrose and starch increased little over the 6 h sampling interval in source leaves of plants grown in 0.4 L containers (Fig. 3, C and D). In contrast, source leaves of plants grown in 5.9 L container volumes accumulated sucrose and starch at rates that were nearly 4 times greater than source leaves of plants grown in 0.4 L containers (Fig. 3, E and F).

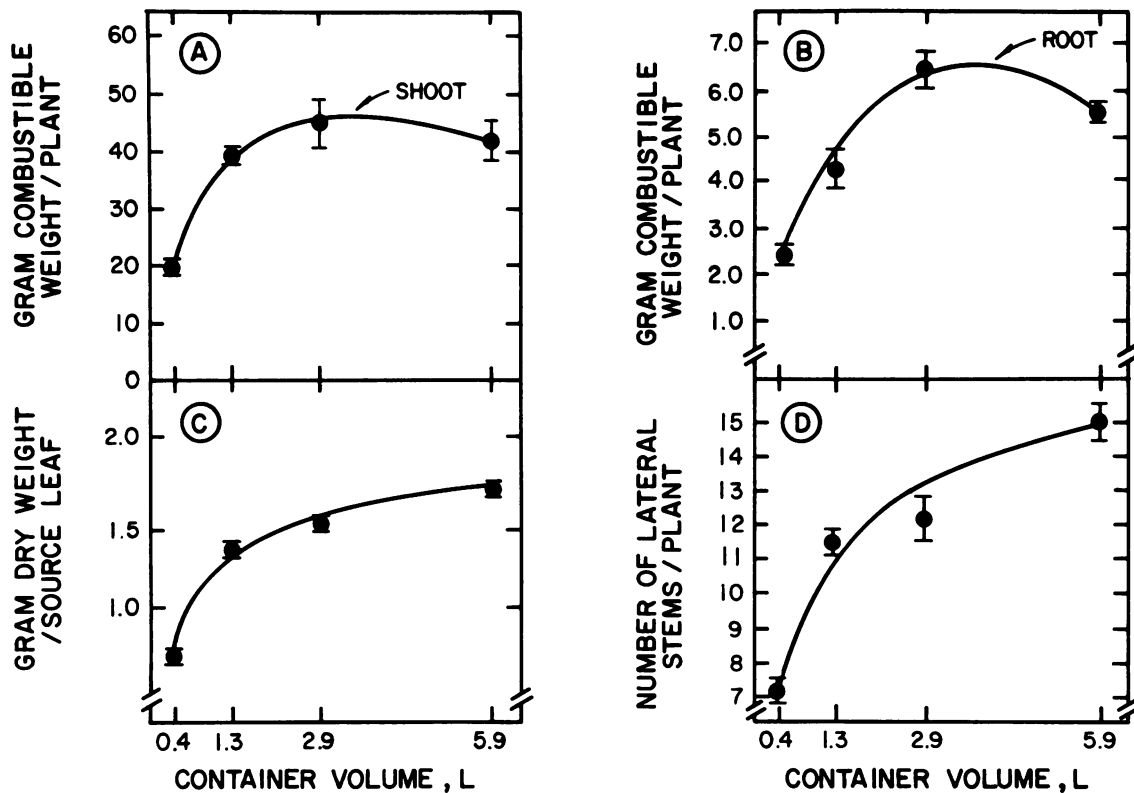


FIG. 1. Influence of four container volumes on vegetative growth of cucumber plants. Each datum represents the mean of four plants and vertical bars are SE.

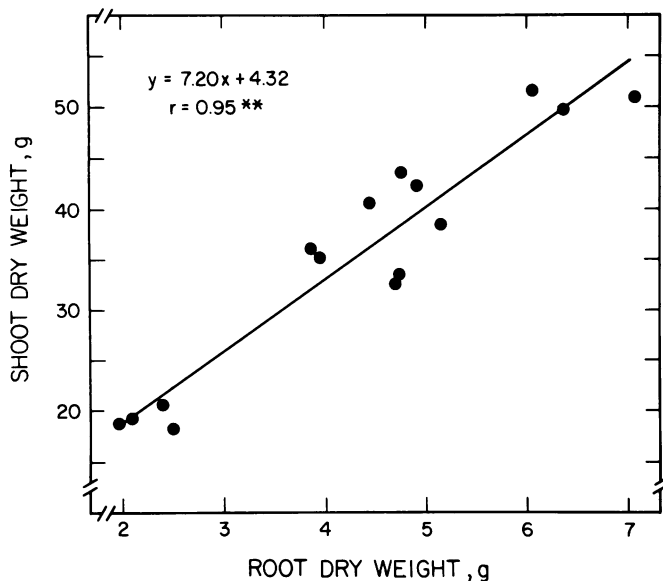


FIG. 2. Relationship between shoot dry weight and root dry weight. Line was obtained by linear regression ($n = 15$).

Although similar diurnal patterns of leaf starch accumulation and mobilization occurred in source leaves of plants grown in 0.4 and 5.9 L container volumes, the magnitude of change over a 24 h period differed greatly (Fig. 4). Starch concentrations per unit area were higher in source leaves of plants grown in 0.4 L containers over the entire day/night cycle. Source leaves of plants grown in 5.9 L containers mobilized nearly $50 \text{ mg} \cdot \text{dm}^{-2}$ over

the 12 h night period. This is in contrast to approximately $15 \text{ mg} \cdot \text{dm}^{-2}$ of starch mobilized from source leaves of plants grown in 0.4 L containers (Fig. 4). Starch concentrations at 1200 on d 1 for leaves of plants grown in 0.4 L containers were significantly ($\alpha = 0.001$) lower than starch concentrations at 1200 on d 2. Collectively, these data suggest a gradual accumulation of starch due to reduced mobilization in source leaves of plants grown in 0.4 L containers.

Concentrations of leaf raffinose and stachyose did not vary significantly over the sampling interval (data not shown). Concentration of galactinol, the galactosyl donor essential for the synthesis of both raffinose and stachyose, was highest in source leaves of cucumber plants grown in 0.4 L container volumes (Fig. 5). In contrast, Gal Syn activity was lowest in leaves of plants grown in 0.4 L containers (Fig. 5). The inverse relationship between Gal Syn activity and galactinol pool size suggests that Gal Syn activity *per se* did not regulate leaf galactinol concentration.

DISCUSSION

Cucumber plants respond to conditions which restrict root growth by limiting both root and shoot growth. Containers that restricted root growth produced cucumber plants with reduced leaf area, fewer lateral stems, and lower root and shoot dry weight (Fig. 1). A coordination between the physical volume in which cucumber roots grew and the ultimate size and morphology of the shoot occurred over each of the container volumes used in the present study (Fig. 3). These results agree with previous studies which indicated that restricting root development under otherwise favorable environmental conditions resulted in dwarf plants which had reduced leaf area and shoot dry weight (2, 9). Availability of essential nutrients did not appear to be a limiting factor in the reduced root and shoot growth of cucumber plants. A similar conclusion was reached in studies with bean and wheat

Table I. Effect of Container Size on Macronutrient and Micronutrient Concentration in Expanded Cucumber Leaves

Pot size	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
<i>L</i>			% dry weight				ppm (dry weight)		
0.4	4.04 ± 0.15	0.45 ± 0.02	3.65 ± 0.33	4.90 ± 0.95	1.06 ± 0.12	204 ± 70	129 ± 14	79 ± 9	9 ± 1
1.3	4.45 ± 0.31	0.75 ± 0.02	4.68 ± 0.20	3.93 ± 0.77	0.73 ± 0.12	113 ± 19	114 ± 23	88 ± 12	10 ± 1
2.9	4.71 ± 0.18	0.79 ± 0.08	4.94 ± 0.49	3.88 ± 0.42	0.73 ± 0.03	95 ± 5	123 ± 20	98 ± 9	10 ± 1
5.9	4.50 ± 0.15	0.72 ± 0.26	4.10 ± 0.55	2.77 ± 1.51	0.59 ± 0.26	103 ± 28	90 ± 16	83 ± 22	9 ± 3

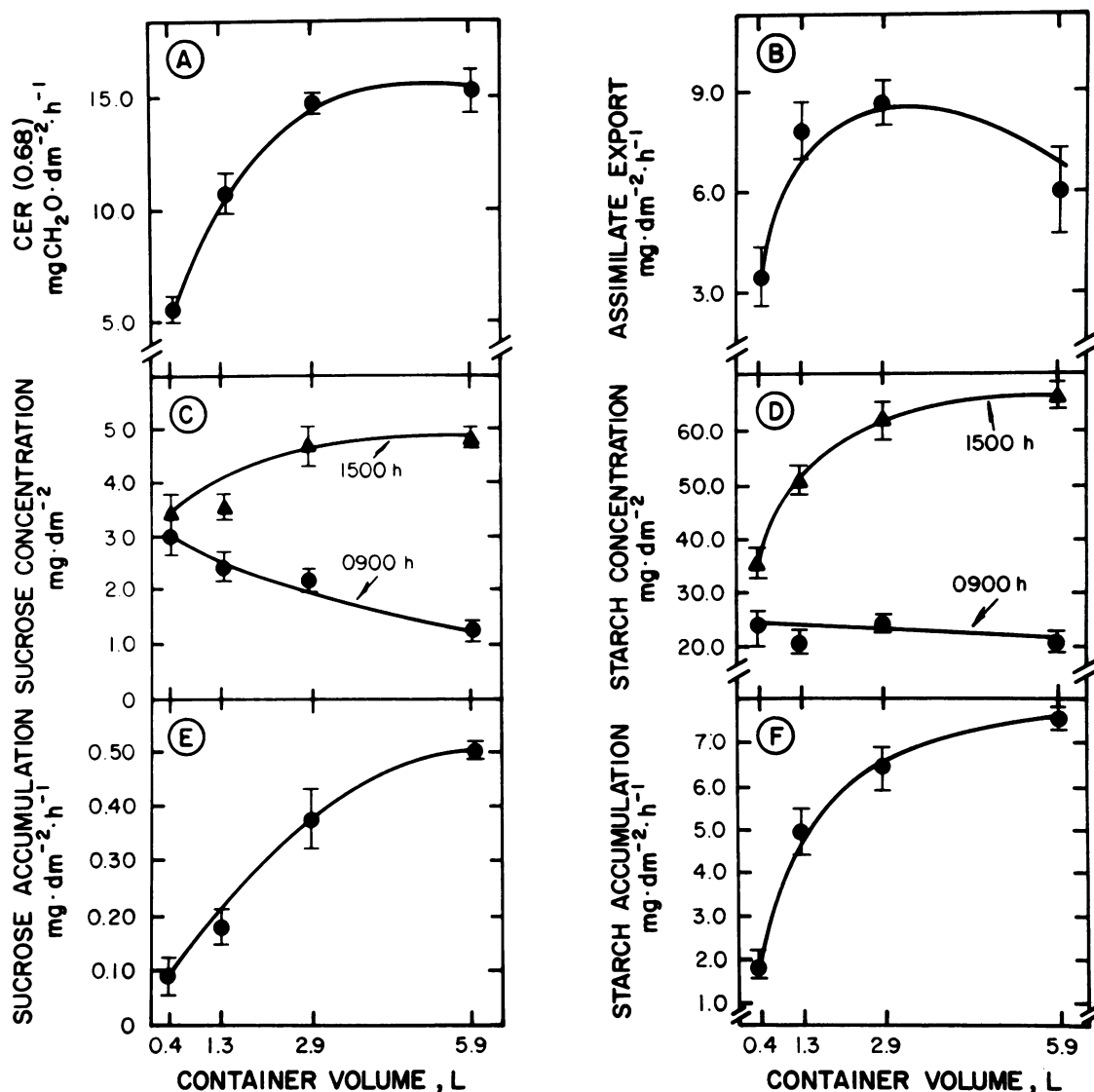


FIG. 3. Influence of four container volumes on shoot carbohydrate metabolism during the photoperiod. Each datum represents the mean of four plants and vertical bars are SE.

plants (2, 9). Increasing available nutrients under a limited root volume did not influence growth of dwarf plants although concentrations of N, P, and K increased within the shoot of dwarf and control plants (2).

Reduced sink demand has been associated with alterations in the partitioning of photoassimilates (4, 15). A change in the balance between assimilate availability and demand requires the establishment of a new equilibrium within biosynthetic pathways (4). Adjustments to rapid alterations in sink demand have been associated with decreased AER and a corresponding increase in starch accumulation (15). Cucumber source leaves appeared to

respond to an environmental condition which restricted root growth by reducing carbon metabolism. Lower rates of photosynthesis were associated with reduced starch accumulation and reduced assimilate export in source leaves of cucumber plants grown in restricted soil volumes (Fig. 3). Over time, the gradual accumulation of starch, due to reduced carbohydrate mobilization, could have contributed to the larger concentrations of starch that were maintained on a diurnal basis in source leaves of plants in 0.4 L containers (Fig. 4) and could have provided a feedback mechanism that reduced carbon metabolism for plants grown in a restricted soil volume. In a recent study, cucumber plants re-

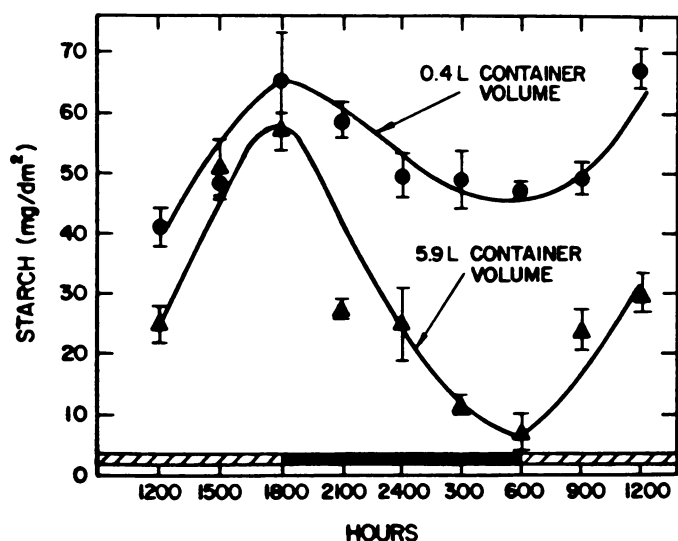


FIG. 4. Influence of 0.4 and 5.9 L container volumes on concentration of starch over a 24 h period. Six leaf 0.5 cm^2 discs samples from three adjacent leaves were removed every 3 h; (◻), daytime; (◼), night. Each datum represents the mean of four plants and vertical bars are SE.

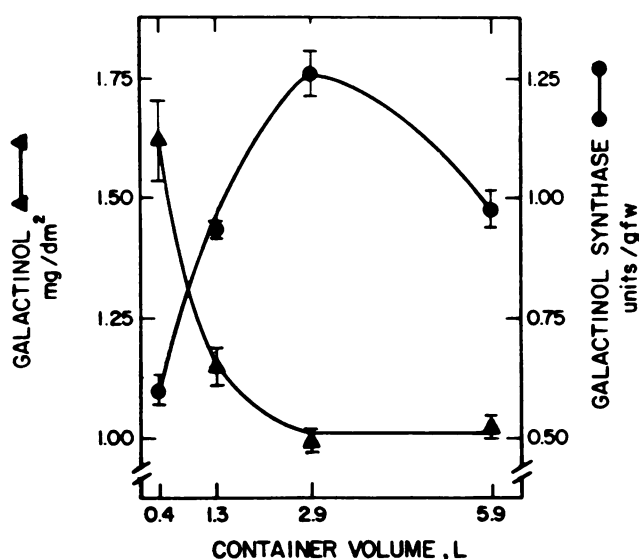


FIG. 5. Influence of four container volumes on concentrations of galactinol and Gal Syn activity. (●), Concentrations of galactinol at 1500 h; (▲), units of Gal Syn activity at 1500 h. A unit is the amount of enzyme which produces $1 \mu\text{mol product} \cdot \text{h}^{-1}$. Each datum represents the mean of four plants and vertical bars are SE.

sponded to the demand of a short (8 h) photosynthetic photoperiod by maintaining the rate of carbon fixation throughout the entire photoperiod, while increasing the rate of starch accumulation at the expense of assimilate export during the latter part of the photoperiod (13). Thus, different strategies are available for altering carbon partitioning in cucumber source leaves in response to specific environmental cues.

It has been suggested that Gal Syn may play an important role in the partitioning of carbon into stachyose (10, 11). On a developmental basis, Gal Syn activity has been associated with raffinose and stachyose biosynthesis in cucumber leaves and maturing soybean seeds (5, 11, 16). Elevated Gal Syn activities have been found in cucumber source leaves with high CER when

plants were grown under light intensities of $650 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ in contrast to low Gal Syn activity in plants grown under $380 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (13). In the present study, high Gal Syn activity was associated with high rates of photosynthesis and assimilate export in source leaves of plants grown in 5.9 L containers (Fig. 5), yet Gal Syn activity did not appear to regulate galactinol pool size in cucumber source leaves, as the two were inversely related across various rooting volumes (Fig. 5). Under conditions that reduced normal root growth, galactinol pool size increased above the steady state concentrations associated with the higher rates of carbon assimilation and export in source leaves of plants in 5.9 L containers. This is consistent with previous studies in which high galactinol concentrations in source leaves of non-fruited cucumber plants were associated with lower rates of photosynthesis, assimilate export, and stachyose biosynthesis than those of fruited plants (10). Galactinol accumulation in cucumber source leaves may be due to diminished stachyose biosynthesis *in situ* due to a reduction in sink demand imposed by restricted root growth.

Acknowledgments—The authors thank Dr. S. C. Huber for use of an Anarad infrared gas analyzer; Dr. P. V. Nelson for greenhouse watering system; Millipore, Waters Chromatography Division, Milford, MA, for HPLC equipment furnished through an equipment grant; and Mrs. H. N. Sox for expert technical assistance.

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